Amendments to the Claims

Please replace all previously pending claims with the listing of claims set forth below. In this new listing of claims, claims 47, 57 and 59 have been amended and claim 51 has been canceled under the provisions of 37 C.F.R. §1.121.

1-8. (canceled)

9. (Previously Amended) The method of claim 47, wherein the subcellular compartment is selected from the group consisting of a cell nucleus, a cytoplasm, a nuclear membrane, a cellular membrane, a mitochondria, an endoplasmic reticulum, a peroxisome and a lysosome.

10. (Previously Amended) The method of claim 47, wherein the biomarker is selected from the group consisting of a protein, a peptide, a nucleic acid, a lipid or a carbohydrate.

11-38. (canceled)

39. (Previously Amended) The method of claim 47, wherein each of the first, the second and the third stain comprises a fluorophore.

40-46. (canceled)

47. (Currently Amended) A computer implemented method for localizing and quantitating a particular biomarker within a ~~plurality of~~ first marker defined subcellular compartment

relative to a second marker defined subcellular compartments present in individual cells of interest contained in a tissue sample comprising:

a) incubating the tissue sample with a first stain that specifically labels ~~a~~ the first marker defined subcellular compartment, a second stain that specifically labels ~~a~~ the second marker defined subcellular compartment, and a third stain that specifically labels the biomarker;

b) obtaining a high resolution image of each of the first, the second, and the third stain in the tissue sample using an upright or inverted optical microscope so as to obtain:

i.) a first image of the first marker defined subcellular compartment;

ii.) a second image of the second marker defined subcellular compartment; and

iii.) a third image of the biomarker;

wherein each image comprises ~~multiple~~ 1024 x 1024pixel locations;

c) reiteratively analyzing each pixel location in the first and the second image so as to assign each such pixel location to the first, the second or neither subcellular compartment based upon an intensity value of the first stain relative to the second stain at that pixel location;

d) analyzing in the third image the pixel locations

assigned to the first or the second subcellular compartment in step (c) so as to identify those pixel locations having an intensity value indicative of the third stain, and determining the total intensity value of the third stain at the pixel locations in each of the first and the second subcellular compartment;

so as to thereby localize and quantitate the biomarker in the first ~~or in~~subcellular compartment relative to the second subcellular compartment.

48. (Previously presented) The method of claim 47, wherein the quantitation of the biomarker present within the first or the second subcellular compartment comprises summing the intensity values of the third stain at the pixel locations within such subcellular compartment and dividing the sum by the number of pixels in such subcellular compartment.

49. (Previously presented) The method of claim 47, wherein a pixel location not assigned to the first or the second subcellular compartment is assigned to a third subcellular compartment.

50. (Previously presented) The method of claim 47, wherein the tissue has a thickness of about five microns.

51. (canceled)

52. (Previously presented) The method of claim 47, wherein the first subcellular compartment is a cellular membrane and the

second subcellular compartment is a cell nucleus.

53. (Previously presented) The method of claim 47, wherein the tissue sample is a fixed tissue section.

54. (Previously presented) The method of claim 47, wherein the first or the second stain reacts with a marker that is selected from the group consisting of cytokeratin, beta catenin, alpha catenin and vimentin.

55. (Previously presented) The method of claim 47, wherein at least one of the first, the second or the third stains comprises a fluorophore selected from the group consisting of 4',6-diamidino-2-phenylindole (DAPI), Cy3 and Cy-5-tyramide.

56. (Previously presented) The method of claim 47, wherein the biomarker is selected from the group consisting of Her-2/neu, estrogen receptor, progesterone receptor and epidermal growth factor receptor.

57. (Currently amended) The method of claim 47, further comprising after step (b) but before step (c) performing a pseudo-deconvolution step comprising:

1.) obtaining an out-of-focus image of each of the first, the second and the third stain in the tissue sample wherein each image has an out-of-focus intensity value for each pixel location; and

2.) subtracting the out-of-focus intensity value for each pixel location from the intensity value at such pixel location in the first, the second and the third images of step (b);

so as to thereby obtain a processed image for each stain,

corrected for background.

58. (Previously presented) The method of claim 47, wherein a mask is applied to the first, the second and the third images.

59. (Currently amended) A computer implemented method for localizing and quantitating a particular biomarker within a ~~plurality of~~ first marker defined subcellular compartment relative to a second marker defined subcellular compartment~~s~~ present in individual cells of interest contained in a tissue sample comprising:

a) incubating the tissue sample with a first stain that specifically labels a first marker defined subcellular compartment, a second stain that specifically labels a second marker defined subcellular compartment, and a third stain that specifically labels the biomarker;

b) obtaining a high resolution image of each of the first, the second, and the third stain in the tissue sample using an upright or inverted optical microscope so as to obtain:

i~~.~~) a first image of the first marker defined subcellular compartment;

ii~~.~~) a second image of the second marker defined subcellular compartment; and

iii~~.~~) a third image of the biomarker~~;~~,

wherein each image comprises ~~multiple~~ 1024 x 1024pixel locations;

c) (1) determining the first and second stain intensity in each of the pixel locations in the first and the second image and assigning those pixel locations having an intensity indicative of:

i~~.~~) the first stain only, to the first compartment;

ii~~.~~) the second stain only, to the second compartment;

iii~~.~~) both the first and the second stain, to the compartment for which the stain intensity is greater or to neither compartment if the stain intensity is substantially equal;

(2) reiteratively analyzing the first and the second stain intensity in each of the pixel locations assigned to each of the first compartment and the second ~~the other~~ compartment ~~to assess spillover~~ and reassigning each pixel location based on a weighted ratio of the first stain intensity relative to the ~~to~~ second ~~compartment~~ stain intensity to reach a 95% degree of accuracy in the assignment of the pixel location;

d) analyzing in the third image the pixel locations assigned to the first subcellular compartment or the second subcellular compartment in step (~~d~~c) so as to identify those pixel locations having an intensity value indicative of the third

stain, and determining the total intensity value of the third stain at the pixel locations ~~in~~ assigned to each of the first and second subcellular compartment;

so as to thereby localize and quantitate the biomarker in the first ~~or in~~subcellular compartment relative to the second subcellular compartment.